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Report on the Analysis of Honey

CARBOHYDRATES IN HONEY

For several years it has been the recommendation of Subcommittee D that the selective adsorption method for determination of the sugars of honey (1) be studied collaboratively. One limited study was reported (2). The Associate Referee has been attempting to initiate such a study but has not been able to secure outside collaborators. An extensive analytical study of the composition of American honey, including over 500 samples of all important floral types and blends, is now under way in this laboratory. Carbohydrates are being determined by this procedure.

Four check samples of honey have been analyzed for carbohydrates by three analysts in this project. The samples were stored in the frozen state to minimize composition changes of the honeys that might be due to enzyme or acid action. The procedure used is that previously described (1), except that the equations for calculating fructose and glucose values are now revised and corrected for overall yields from recovery data with known sugars. The results are given in Table 1.

The problem of selecting a suitable charcoal adsorbent and finding the corresponding proper alcohol content for eluents is under study. Some information on these questions is in an earlier publication (1). The same question was examined by Patterson and Savage (3) who developed a selective desorption procedure for starch conversion products; they fractionated the eluate and determined sugars in each portion. By constructing histograms of sugar concentration versus eluate volume they could compare the performance of any carbon to that of any others they had found satisfactory and adjust the elution conditions to give comparable results. This essentially was the approach in the development of the honey method and can be used to select charcoal samples and adjust alcohol concentrations of eluants to give suitably quantitative recovery. In developing the procedure, that alcohol content of solvent was selected which would completely elute the sugars in a total volume of 200 ml; the method uses 250 ml volumes. Evaporation and weighing of residues, as outlined below, appears satisfactory. Efficiency of the separations in practice is easily monitored by routine paper chromatography of fractions.

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Selective Adsorption Method

Principle

By adsorption of honey sample on charcoal column, followed by elution into monosaccharide, disaccharide, and higher sugar fractions, interference of disaccharides in dextrose and levulose detns is eliminated. Elution is by progressively higher EtOH concns, followed by detn of individual monosaccharides, sucrose, reducing disaccharides collectively as maltose, and trisaccharides and higher sugars collectively after hydrolysis.

Preparation and Standardization of Adsorption Column

Column is 22 mm o.d. × 370 mm long, with 1 L spherical section and 35/20 spherical ground joint at top. Adsorbent is 1 + 1 mixt. of Darco G-60 charcoal and rapid filter-aid (Celite 545 or Dicalite 4200). Insert glass wool plug, wet from below, and add enough dry adsorbent to the dry tube (23–26 cm) to compress to 17 cm when vacuum is applied with *gentle* tapping of column. Remove excess charcoal from walls of column, and add filter-aid layer at top with *gentle* packing (1–1.5 cm). Wash column with 500 ml H_2O and 250 ml 50% EtOH, and let stand overnight with 50% EtOH on it. Flow rate should be 5.5–8.0 ml/min. with H_2O at 9 lb/sq.in. pressure. Slower flow rates delay analyses excessively.

Alcohol content of eluting solns must be adjusted to retentive power of charcoal used. Wash column EtOH-free with 250 ml H_2O, quantitatively add 10 ml soln of 1.000 g anhyd. glucose to top, and draw it into column with

Table 1. Collaborative analyses of four honey samples by selective adsorption

Sample	Analyst	Glucose, %	Fructose, %	Sucrose, %	Maltose, %	Higher Sugars, %
222	MS	27.27	36.40	0.58	10.58	2.02
	JW	26.47	37.20	0.94	10.02	2.20
	IK	27.69	37.36	0.73	10.79	2.23
260	MS	33.00	41.00	1.52	7.02	0.82
	JW	32.44	40.61	1.48	6.98	1.00
	IK	33.04	40.92	1.59	7.30	0.78
264	MS	28.62	38.19	0.48	7.99	0.89
	JW	28.38	38.68	0.80	7.46	0.96
	IK	28.40	38.50	0.79	7.76	0.88
268	MS	33.95	39.00	1.06	4.87	0.64
	JW	33.44	39.39	1.28	5.33	0.61
	IK	33.66	38.21	1.08	5.33	0.59
Std. Dev.		0.38	0.42	0.14	0.28	0.08

suction (do not let dry). Add 300 ml H_2O to top, break suction, apply pressure (10 lb/sq.in. max.), and collect eluate in five 50 ml portions in tared beakers. Include 10 ml from sample introduction in first 50 ml fraction. Evap. fractions on steam bath, dry in vac. oven at 80–100°, and weigh.

Decant remaining H_2O from top of column, pass 50 ml 50% EtOH and then 250 ml H_2O thru column, and repeat chromatography, using 1.000 g anhyd. glucose in 10 ml 1% EtOH, washing with 250 ml 1% EtOH as above. Select as solvent *A* that which removes glucose in 150 ml. Repeat chromatography with 2% EtOH if necessary.

Wash column with 250 ml H_2O and then 20 ml 5% EtOH. To top, add 10 ml 5% EtOH soln contg 100 mg maltose and 100 mg sucrose. Elute as above with 250 ml 5% EtOH, weighing evapd 50 ml portions of filtrate. Repeat, if necessary, with 7%, 8%, and 9% EtOH to find solvent *B* that will elute at least 98% disaccharides in 200 ml. Solvent *A* previously selected must not elute disaccharides. Combinations found satisfactory with various charcoals are 1%, 7%; 2%, 8%; 2%, 9%. At conclusion, pass 100 ml 50% EtOH thru column, and store under layer of this solvent.

Preparation of Fractions

Wash column with 250 ml H_2O and decant any supernatant. Pass 20 ml solvent *A* thru column, and discard. Dissolve 1 g sample in 10 ml solvent *A* in 50 ml beaker. Transfer sample (using long-stem funnel) onto column, and force into column. Use 15 ml solvent *A* to rinse beaker and funnel, and add to column. Collect all eluate, beginning with sample introduction, in 250 ml vol. flask. Add 250 ml solvent *A*, and collect exactly 250 ml total (fraction *A*-monosaccharides). Decant excess solvent from top, add 265–270 ml solvent *B*, and collect 250 ml in vol. flask (fraction *B*-disaccharides). Decant excess, add 110 ml 50% EtOH (solvent *C*), and collect 100 ml in vol. flask (fraction *C*-higher sugars). Mix each fraction thoroly. Column may be stored indefinitely, outlet closed, under 50% EtOH. Discard after 8 uses.

Fructose

Reagents

(a) *Iodine soln.*—0.05*N*. Dissolve 13.5 g pure I in soln of 24 g KI in 200 ml H_2O, and dil. to 2 L. Do not stdze.

(b) *Sodium hydroxide soln.*—0.1*N*. Dissolve 20 g NaOH and dil. to 5 L.

(c) *Sodium hydroxide soln.*—1*N*. Dissolve 41 g NaOH in H_2O and dil. to 1 L.

(d) *Sulfuric acid soln.*—1*N*. Add 56 ml H_2SO_4 to H_2O and dil. to 2 L.

(e) *Sulfuric acid soln.*—2*N*. Add 56 ml H_2SO_4 to H_2O and dil. to 1 L.

(f) *Sodium sulfite soln.*—1%. Dissolve 1 g Na_2SO_3 in 100 ml H_2O. Make fresh daily.

(g) *Starch soln.*—1% (freshly prepd). *See* 2.64(d).

(h) *Bromcresol green soln.*—Dissolve 150 mg bromcresol green in 100 ml H_2O.

(i) *Shaffer-Somogyi reagent.*—Dissolve 25 g each anhyd. Na_2CO_3 and Rochelle salt in ca 500 ml H_2O in 2 L beaker. Add 75 ml of soln of 100 g $CuSO_4.5H_2O$/L, thru funnel with tip under surface, with stirring. Add 20 g dry $NaHCO_3$, dissolve, and add 5 g KI. Transfer soln to 1 L vol. flask, add 250 ml 0.100*N* KIO_3 (3.567 g dissolved and dild to 1 L), dil. to vol.,

and filter thru fritted glass. Age overnight before use (4).

(j) *Iodide-oxalate soln.*—Dissolve 2.5 g KI and 2.5 g K oxalate in 100 ml H_2O. Make fresh weekly.

(k) *Sodium thiosulfate std soln.*—0.005*N*. Prep. from stdzd stock 0.1000*N* soln. (*See* 41.35.) Make fresh daily.

Determination

Pipet 20 ml fraction *A* into 200 ml vol. flask. Add 40 ml 0.05*N* I soln by pipet, then with vigorous mixing add 25 ml 0.1*N* NaOH over 30 sec. period, and immediately place flask in 18±0.1° water bath. Exactly 10 min. after alkali addn, add 5 ml 1*N* H_2SO_4 and remove from bath. Exactly neutralize I with Na_2SO_3 soln, using 2 drops starch soln near end point. Back-titr. with dil. I if necessary. Add 5 drops bromcresol green and exactly neutralize soln with 1*N* NaOH; then make just acid to indicator. Dil. to vol. and det. reducing value of 5 ml aliquots by Shaffer-Somogyi method: place 5 ml in 25 × 200 mm test tubes, add 5 ml Shaffer-Somogyi reagent, and mix by swirling. Place in boiling H_2O bath and cap with funnel or bulb. After 15 min., remove to running H_2O cooling bath with care, and cool 4 min. Carefully remove caps, and add, down side, 2 ml iodide-oxalate soln and then 3 ml 2*N* H_2SO_4. (Do not agitate soln while alk.) Mix thoroly, seeing that all Cu_2O is dissolved. Return to cold H_2O and let stand 5 min., mixing twice in this period. Titr. in tube with 0.005*N* $Na_2S_2O_3$ and starch indicator. (Magnetic stirrer is most suitable for purpose.) Make duplicate blanks and detns. Deduct titrn from that of blank and calc. fructose:

$$\% \text{ fructose} = \frac{500\,[(\text{titer} \times 0.1150) + 0.0915] \times 100}{\text{mg sample}}$$

Fructose correction for dextrose detn = f.c. = [(titer × 0.1150) + 0.0915] × 40. Bracketed quantity is mg fructose in 5 ml aliquot, valid between 0.5 and 1.75 mg fructose.

Dextrose

Reagents

Sodium thiosulfate soln.—0.05*N*. Prep. from stdzed stock 0.1000*N* soln (*see* 41.35).

Determination

Pipet 20 ml fraction *A* into duplicate 250 ml erlenmeyers. Evap. to dryness on steam bath in air current. Add 20 ml H_2O, pipet 20 ml 0.05*N* I, and as in fructose detn, add 25 ml 0.1*N* NaOH slowly, and immediately place in 18±0.1° H_2O bath. Exactly 10 min. from end of alkali addn, add 5 ml 2*N* H_2SO_4, remove from bath, and titr. with 0.05*N* $Na_2S_2O_3$, using starch soln. Make duplicate blanks, using H_2O. Subtract titrn value from that of blank, and calc. glucose:

$$\% \text{ glucose} = \frac{56.275\,[\text{titer} - (0.01215 \times \text{f.c.})] \times 100}{\text{mg sample}},$$

where f.c. = fructose correction from fructose detn. Equation is valid over range 10–50 mg dextrose in 20 ml. In presence of glucose, 1 mg fructose requires 0.01215 ml 0.05*N* $Na_2S_2O_3$, in range 15–60 mg fructose.

Reducing Disaccharides as Maltose

Determination

Pipet 5 ml aliquots of fraction *B* into 25 × 200 mm test tubes, and add 5 ml Shaffer-Somogyi reagent. Det. reducing value as in fructose detn, except boil tubes 30 min. Value for 15 min. H_2O blank may be used here. Calc. % reducing disaccharides as maltose:

$$\% \text{ "maltose"} = \frac{50[(\text{titer} \times 0.2264) + 0.075] \times 100}{\text{mg sample}}$$

Maltose correction for sucrose detn = m.c. = maltose titer × 0.92. Bracketed quantity is mg maltose in 5 ml aliquot, valid between 0.15 to 3.80 mg maltose. Reducing value of maltose at 15 min. is 92% of final value.

Sucrose

Reagents

(a) *Hydrochloric acid soln.*—6*N*. Add 250 ml HCl to H_2O and dil. to 500 ml.

(b) *Sodium hydroxide soln.*—5*N*. Dissolve 103 g NaOH in H_2O and dil., after cooling, to 500 ml.

Determination

Pipet 25 ml fraction *B* into 50 ml vol. flask. Add 5 ml 6*N* HCl and 5 ml H_2O. Mix, let stand in 60° H_2O bath 17 min., cool, and neutralize to bromcresol green with 5*N* NaOH (Polyethylene squeeze bottle is excellent for holding and delivering alkali.) Adjust to acid color of indicator, using 2*N* H_2SO_4 to correct over-run. Dil. to vol. and det. reducing value of 5 ml aliquots by Shaffer-Somogyi detn as for fructose. Subtract titrn from blank, and calc. sucrose by reference to curve constructed from following table (5):

Sucrose in 5 ml Aliquot Oxidized, mg	*0.005N $Na_2S_2O_3$ Required, ml*
0.255	1.75
0.502	3.95
1.004	8.72
1.260	11.28

From curve obtain S_1 = sucrose equiv. to maltose correction (see above for maltose) and S_2 = sucrose equiv. of sucrose titer.

$$\% \text{ sucrose} = \frac{50\ (2S_2 - S_1) \times 100}{\text{mg sample}}$$

Higher Sugars as "Dextrin"

Pipet 5 ml aliquots of fraction *C* into 50 ml vol. flasks. Add 5 ml 6*N* HCl and 5 ml H_2O, and heat in boiling H_2O bath 45 min. Cool, neutralize as for sucrose, dil. to vol., and det. reducing value by Shaffer-Somogyi detn as for fructose. Subtract titrn value from blank and obtain glucose equiv. from curve constructed from data below:

Glucose, mg	*Titer, ml*
0.05	0.20
0.10	0.60
0.25	1.85
0.50	4.00
1.00	8.50
2.00	17.60

$$\% \text{ higher sugars} = \frac{40(\text{glucose equiv.}) \times 100}{\text{mg sample}}$$

Notes

For most accurate work, Shaffer-Somogyi values must check within 0.04 ml. Calibration of entire procedures, including column using known synthetic mixts of dextrose, levulose, sucrose, maltose, and raffinose (corrected for moisture) is recommended for critical work. Efficiency of column sepn may be checked by paper chromatography of fractions *A, B,* and *C* as described in *Detection of Commercial Glucose* (p. 347).

DIASTASE VALUE OF HONEY

(with F. W. PAIRENT)

The determination of the diastatic activity of honey has been a matter of great importance in Europe for years. The lability of the enzyme to heat has been used in several countries as a means of determining whether a honey has been exposed to excessive heating. Such heating is highly undesirable for table honey in their view.

Although occasional shipments of American honey have been declared unacceptable to European importers because of low diastase values, relatively little attention has been given to the determination of diastatic activity of honey in this country. Procedures proposed (6–8) have been modifications of the Gothe method (9, 10) which has been modified recently in Germany (11). The present A.O.A.C. procedure, **29.113,** was developed by Auzinger in 1910 (12) from a test for milk diastase published in 1907 by Koning. It was superseded in Germany many years ago by the Gothe test.

Recently Schade, Marsh, and Eckert (13) have described a method for diastase determination in honey in which a photoelectric photometer is used to measure starch-iodine color. This method appears more reproducible than the modified Gothe test, provides a continuous scale of diastase values (which the Gothe method does not), and is suited for routine use. As originally described, a starch solution is degraded by the amylase(s) in a honey solution under standard conditions. An arbitrary end point is specified, defined originally as a reading of 150 for the iodine-treated solution in the Klett-Summerson colorimeter in a dilution that gave a Klett value of 850–900 with the original starch solution after addition of iodine. This is a reduction of absorbance to about 17% of the original value.

We have studied this procedure with a view to using it in the analytical survey described above. We used the procedure exactly as Schade described it. Results did not agree with those by the modified Gothe method, but were considerably lower.

The procedure was brought into harmony with the Gothe method by changing the starting density and the end point specification. Since the Gothe method has been used for diastase for years, it is important that any new method gives values that are comparable. Because the end point is arbitrarily defined, the determination of diastatic value is empirical and does not produce absolute values. Hence calibration of the new procedure to give concordant results with the older one is permissible.

Recent inquiry has shown that workers at

the Institut für Honigforschung, Bremen, Germany, use the Schade method (14). They independently reduced the starting density to obtain results concordant with the Gothe procedure.

Table 2. Collaborative analysis of 20 honey samples for diastase number

Sample	Gothe Method[a]	Schade Method Coll. A	Coll. B
24	6.5	6.5	6.9
421	23.8	27.3	28.1
459	10.9	11.5	12.7
341	8.3	11.2	10.9
460	13.9	13.3	12.3
416	17.9	16.9	19.2
77	29.4	33.0	32.0
240	29.4	34.5	32.5
221	29.4	34.1	34.5
350	50.0	52.5	46.2
3327	<38.5	38.5	38.2
3390	23.8	25.5	24.3
3385	13.9–17.9	14.1	15.9
3395	13.9	13.0	14.1
3391	<29.4	27.5	28.8
3392	8.3–10.9	10.8	9.5
3402	>10.9	7.5	11.7
3329	<38.5	35.7	36.5
3388	<23.8	22.1	22.6
3394	>23.8	24.3	24.1

[a] Samples 24–350 by Coll. A; remainder by Coll. B.

Table 2 shows the results of the determination of diastatic activity of 20 honey samples by two methods, modified Gothe (11) and Schade, as described in this report, at two laboratories. Gothe values were not determined by both collaborators for each sample. The agreement for the Schade values between laboratories is in general encouraging. The range of differences is somewhat larger than desired, though it is within one Gothe "step" except for sample 3402. Values obtained by Collaborator A average 99.8% of those of Collaborator B, with 3402 excluded; 98.0%, including all samples. Further collaborative work is planned. The procedure is described below essentially as presented by Schade, Marsh, and Eckert (13).

During routine determinations of diastatic activity of samples in the analytical survey referred to above, one analyst made five determinations on one honey on five different days. The results are given in Table 3 and indicate the satisfactory reproducibility of the method.

Table 3. Reproducibility of Schade diastase determination

Date	Sample Wt	Starting Absorbance	End Point, min.	Diastase No.
20	5.006	0.765	15.5	19.4
22	10.040	0.760	15.5	19.4
25	4.995	0.770	14.5	20.7
26	4.970	0.768	15.5	19.4
27	5.035	0.772	14.5	20.7

Diastatic Activity

Principle

Buffered sol. starch-honey soln is incubated and time required to reach specified end point is detd by photoelec. photometer. Results are expressed as ml 1% starch hydrolyzed by enzyme in 1 g honey in 1 hr.

Apparatus

(a) *Reaction vessel.*—Attach side-arm, 18 × 60 mm, to 18 × 175 mm test tube. Lower side of side-arm is attached 100 mm from bottom of tube, making 45° angle with lower portion of tube.

(b) *Photoelectric colorimeter.* — Equipped with 660 mμ red filter, or 600 mμ interference filter.

Reagents

(a) *Iodine stock soln.*—Dissolve 8.80 g resublimed I_2 in 30–40 ml H_2O contg 22.0 g KI, and dil. to 1 L with H_2O.

(b) *Iodine soln.*—0.0007*N*. Dissolve 20 g KI and 5.00 ml I soln, (a), in H_2O and dil. to 500 ml. Make fresh every second day.

(c) *Acetate buffer.*—pH 5.3 (1.59*M*). Dissolve 87 g $NaOAc.3H_2O$ in 400 ml H_2O, add ca 10.5 ml HOAc in H_2O, and dil. to 500 ml. Adjust pH to 5.30 with NaOAc or HOAc, if necessary.

(d) *Sodium chloride soln.*—0.5*M*. Dissolve 14.5 g NaCl in H_2O and dil. to 500 ml.

(e) *Starch soln.*—Weigh 2.000 g sol. starch (Pfanstiehl, reagent grade, Improved Lintner Method or equiv.) and mix with 90 ml H_2O. Rapidly bring to boil, swirling soln as much as possible. Boil gently 3 min., cover, and let cool to room temp. Transfer to 100 ml vol. flask and dil. to vol. Observe procedure closely to limit variation in blank starch-I absorbance values.

Standardization

Pipet 5 ml starch soln into 10 ml H_2O and mix well. Pipet 1 ml of this soln into several 50 ml graduated cylinders contg 10 ml of the

dil. I soln. Mix well, and det. H_2O diln necessary to produce absorbance value of 0.760±0.02 in photometer-test tube (or cell) combination to be used. This is std diln for starch prepn used. Repeat when changing starch source.

Determination

Weigh 5 g sample into 20 ml beaker, dissolve in 10–15 ml H_2O and 2.5 ml buffer soln, and transfer to 25 ml vol. flask contg 1.5 ml NaCl soln. Dil. to vol. (Soln must be buffered before adding to NaCl soln.)

Table 4. Detection of commercial glucose in honey by chromatographic method

Sample		Coll. 1	Coll. 2	Coll. 3
A	Tulip Poplar Honey, 20% corn sirup	+	+	+
B	Tulip Poplar Honey	−	−	−
C	Pine Honeydew	−	−	−
D	Vetch Honey, 24% corn sirup	+	+	+
E	Pine Honeydew, 21% corn sirup	+	+	+
F	Oak Honeydew	−	−	

Pipet 5 ml starch soln into side arm of reaction tube and 10 ml sample soln into bottom of tube, with care not to mix. Place tube in H_2O bath 15 min. at 40±0.2°; then mix contents by tilting tube back and forth several times. Start stopwatch. At 5 min., remove 1 ml aliquot with pipet and add rapidly to 10.00 ml dil. I soln in 50 ml graduated cylinder. Mix, dil. to previously detd vol., and det. absorbance in photoelec. photometer. Note time from mixing of starch and honey to addn of aliquot to I as reaction time. (Place 1 ml pipet in reaction tube for reuse when later aliquots are taken.) Continue taking 1 ml aliquots at intervals until absorbance value of <.235 is obtained.

The 5 min. value gives an approximation of end point as follows:

Absorbance	*End Point, min.*
0.7	>25
0.65	20–25
0.6	15–18
0.55	11–13
0.5	9–10
0.45	7–8

Calculation

Plot absorbance vs. time on rectilinear paper; draw straight line thru starting absorbance and as many points as possible. From graph det. time dild reaction-I mixt. reaches absorbance of 0.235. Divide 300 by this time to obtain diastase no. (DN).

Notes

A 5-min. reading is sufficient for detg end point of sample with high DN if another value is taken soon after. In samples with low DN, no readings need be taken till within few min. of end point. Only 2 such readings are needed. The 5 min. value will not accurately predict low DN.

COMMERCIAL GLUCOSE IN HONEY

The procedure for detection of commercial glucose in honey given in *Official Methods of Analysis,* **29.107**, was described by Beckmann in 1896. Browne gave considerable attention to this problem in his Bulletin 110 (15) because it was of immediate importance in the early 1900's. Browne did point out, as noted in method **29.107**, that "there are some glucose sirups of high conversion, and certain maltose sirups which do not give any reaction with iodine." He further pointed out that other confirmatory tests were required to demonstrate this type of adulteration.

With the advent of chromatography, new and sensitive procedures are available for this purpose. A procedure has been developed for distinguishing between starch maltodextrins and honeydew or honey "dextrin" or higher sugars. The higher saccharides of honey and of honeydew contain fructose, whereas maltodextrins contain only glucose. By use of suitable chromogenic reagents, the presence of the maltodextrin series can be shown on the paper chromatogram in mixture with honey or honeydew sugars. The procedure can detect corn sirup in honey or honeydew. It is being submitted to collaborative study. Taufel and Greiner (16) have developed a somewhat similar procedure for detection of starch sirup in jelly, marmalade, and artificial honey. They subjected the sample without preliminary precipitation to paper chromatography (four-time multiple development with one solvent and two-time with a second) and used the same color reagent described here.

Six samples were prepared for collaborative examination. Their composition and the results obtained by three collaborators are given in Table 4. Collaborator 3 ranked

Sample F below Samples A, D, and E in intensity. Collaborator 2 noted that this sample gave a "brownish streak darker than the background" and interpreted it correctly, according to the procedure, as negative. Samples B and C gave no color in this region of the chromatogram. Collaborators 1 and 2 applied the A.O.A.C. test, **29.107**, to the six samples and obtained negative results for all.

Commercial Glucose

Reagent

Aniline-diphenylamine chromogenic reagent.—Dissolve 500 mg diphenylamine.HCl and 0.55 ml redistd aniline in 50 ml acetone. Add 5 ml 85% H_3PO_4. Prep. fresh daily. (17)

Preparation of Sample

Dil. sample with H_2O (1 + 1). To 0.5 ml in small centrifuge tube (11 × 100 mm test tube) add 4 ml absolute EtOH, shake, and centrifuge. Decant clear or slightly cloudy supernatant, dissolve ppt in 0.5 ml H_2O, reppt with 4 ml absolute EtOH, and centrifuge. Decant, and dissolve ppt in 0.1 ml H_2O. Apply 1 microliter to origin of paper chromatogram, as well as control spots of authentic honey and/or honeydew and corn sirup treated as above.

Chromatography

Ascending or descending is satisfactory. Suitable solvent for latter is *n*-propanol-EtOAc-H_2O, 7:1:2. Equilibrate 45 min. and irrigate at least 40 hr, letting solvent drip from serrated lower edge of paper. For shorter ascending use (ca 6 hr) roll paper into cylinder, staple edges, and set in cylindrical jar, using isoamyl alcohol-pyridine-H_2O, 7:7:6. To obtain increased resolution, dry paper and repeat irrigation 1 or more times.

Irrigate with suitable solvent, remove, and dry paper chromatogram. Dip in chromogenic reagent, let acetone evap., and heat at 85–95° ca 5–8 min. until control spots of corn sirup treated as above give blue color. Honey or honeydew sample contg 5% of commercial glucose shows series of blue maltodextrin spots of low R_F, converging to origin. Honey and honeydew dextrin spots are distinctly brown or gray, not blue. If paper is heated excessively, both honey dextrin spots and maltodextrin spots will approach same shade of gray.

HONEY ACIDITY

The improved procedure recently described (18) for determination of the acidity (and lactone content) is also being used in the analytical survey described above. Results to date indicate that the lactone content may be of considerable importance in evaluating honey; the procedure will be submitted for collaborative study shortly.

PREPARATION OF HONEY SAMPLES

The procedure given in *Official Methods of Analysis* for preparation of sample, **29.92**, requires that the honey sample, if granulated, be heated *with stopper loose* in a water bath at not over 50° with occasional stirring until the sugar crystals dissolve. Two objections may be made to this procedure: the loose stopper permits loss of moisture, and the 50° temperature is not high enough to dissolve the dextrose hydrate of many samples. In a study in which several hundred samples were liquefied, even 60°C for 30 minutes was not sufficient; if a sample was not liquefied after 30 minutes at 60° the bath temperature was routinely increased to 65° and frequently an additional 1 or 2 hours or more was required. The relative merits of using a long time at 50° or a shorter time at 60 or 65° might be resolved in favor of the latter, provided (a) the sample is removed from the bath as soon as it is liquid and immediately cooled; (b) the sample is tightly stoppered but the top not submerged; and (c) portions for determination of diastatic activity are removed before the sample is heated. Here precautions must be taken to obtain a representative portion of a semi-solid sample.

HONEY COLOR STANDARDS

The U.S. Department of Agriculture has developed a color comparator containing permanent glass color standards (19, 20) and has adopted it for official color grading of honey. It is desirable that a description of the standards and the procedure for their use appear in *Official Methods of Analysis*.

Recommendations

It is recommended*—

(1) That the selective adsorption method for determination of the sugars of honey as

* For report of Subcommittee D and action of the Association, see *This Journal*, 42, 29, 30 (1959).

described in the Associate Referee's report be adopted as first action.

(2) That the Schade method for determination of the diastatic activity of honey as described in the Associate Referee's report be adopted as first action.

(3) That the chromatographic procedure for detection of commercial glucose in honey as described in the Associate Referee's report be adopted as first action.

(4) That the method for determining the free lactone and total acidity in honey, previously described in *This Journal,* **41**, 194 (1958), be submitted to collaborative study.

(5) That preparation of sample, **29.92(a)**, be changed to read (line 2): ". . . place container, with stopper tight, in water bath without submerging, and heat 30 min. at 60°; then if necessary heat at 65° until liquefied. Occasional shaking is essential. Mix thoroly, cool rapidly as soon as sample liquefies, and weigh portion for detn. Do not heat honey intended for diastatic detn. If foreign matter . . .".

(6) That the qualitative test for commercial glucose, **29.107**, and the procedure for diastase, **29.113**, be deleted.

(7) That the U.S. Department of Agriculture procedure for determination of color grade of honey be studied collaboratively.

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Preparation of Sample: Mary L. Riethof, Eastern Regional Research Laboratory.

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